IN THE CLAIMS:

Please cancel claims 25 and 38 without prejudice to prosecute them in a subsequent application.

Please amend claims 24, 26, 27, 32, 34, 37, 40 and 41 as shown on the attached "Claim Sheets marked-up to show changes."

A clean copy of all pending claims is attached as "Clean Set of Claims."

REMARKS

The above amendments to the claims find support throughout the specification including at page 14, line 19, to page 16, line 15; page 16, lines 16-27, and page 17 line 5 to page 19, line 7. In view of these amendments and the following remarks supported by the appended Declaration of Neil H. Bander **(Exhibit 1; "Bander Declaration #1")**, submitted during prosecution of the related case serial number 08/838,682, issued as U.S. Patent 6,107,090 on August 22, 2000, and the Declaration of Neil H. Bander **(Exhibit 2; "Bander Declaration #2")** submitted during prosecution of the related case serial number 08/895,914, issued as U.S. Patent No. 6,136,311 on October 24, 2000, the pending claims of the application are in condition for allowance and reconsideration of the rejections in the outstanding office action is respectfully requested.

The claims pending in this application are 24, 26-37 and 39-42 directed to methods of detecting normal, benign hyperplastic, and cancerous prostate epithelial cells using antibodies or antigen binding portions thereof which bind to the extracellular domain of prostate specific membrane antigen present as an integral membrane protein in a living cell.

I. The Section 112, Second Paragraph Rejection

The rejection of claims 24-42 by the Examiner as "unclear in recitation of a 'biological sample'," is respectfully traversed. Applicant directs the Examiner to the specification including at page 15, lines11-24, page 15, line 25 to page 16, line 4 and the Examples, including Example 1 at page 30, wherein the term "biological sample" can include the patient, serum, urine or other body fluids as well as a tissue biopsy. Accordingly, Applicant asserts that the metes and bounds of the term "biological sample" can be determined from the specification.

Claims 24-42 are rejected by the Examiner under 35 U.S.C. 112, second paragraph as "unclear in the recitation of a 'biological agent'." The claims have been amended to substitute the term "antibody or antigen binding portion thereof" for the term "biological agent," without prejudice to pursue the "biological agent" claims in a subsequent case. Applicant respectfully asserts that this amendment obviates the Examiner's rejection on this ground.

Claims 34-36 are rejected by the Examiner as "unclear in the recitation 'wherein an antibody is used in carrying out said method." Applicant has amended claim 34, which now depends on claim 24 and asserts that it is clear to what the recitation of "antibody" refers.

Claim 38 has been cancelled without prejudice to pursue this invention in a subsequent related case and the Examiner's rejection is therefore obviated.

II. The Section 112, First Paragraph Rejection

Claims 24-42 are rejected by the Examiner under 35 U.S.C. 112, first paragraph "because the specification, while being enabling for an *in vitro* method of detection of LNCaP cells or prostate tissue using the antibodies E99, J415, J533, and J591, does not reasonably provide enablement for methods of detecting normal, benign hyperplastic and cancerous prostate epithelial cells including *in vivo* imaging studies, using any agent which binds the extracellular region of PSMA." The

Examiner asserts that "the specification fails to provide sufficient guidance and objective evidence to enable one skilled in the art to predictably detect normal, benign hyperplastic and cancerous prostate epithelial cells, including *in vivo* detection, using agents or antibodies that bind the extracellular domain of PSMA and target tumor vasculature"; the "instant demonstration that new antibodies E99, J415, J533, and J591 bind to vasculature in fixed tissue does not render it predictable that the new antibodies are binding to PSMA..."; "there is no direct objective evidence supplied that the antibodies are binding the same antigen [as 7E11]"; "it is unpredictable and would require undue experimentation to bind agents to PSMA which are also internalized"; and the specification "provides insufficient objective evidence that antibodies to the PSMA extracellular domain, or antibodies E99, J415, J533, and J591 effectively target tumor vasculature *in vivo*" and cites Jain, R.K. et al. As detailed below, Applicant respectfully traverses these objections.

A. The Method to Prepare Antibodies Described in the Application that bind to the External Domain of PSMA is Reproducible.

As asserted successfully during prosecution of the allowed parent application (U.S. Patent No. 6,107,090) of the instant application, the specification of the application describes a novel method whereby one skilled in the art could reproducibly prepare the monoclonal antibodies of the present invention which bind to the extracellular domain of PSMA present as an integral membrane protein of a living cell. As developed below, Applicant's use of the same method to generate four (4) different antibodies from two completely separate immunization and fusion experiments is compelling evidence of enablement.

The procedures for producing these antibodies are fully described in the examples of the present application. In particular, the four antibodies described in the application (i.e. E99, J415, J533 and J591) were prepared as set forth in Example 3. Specifically, BALB/c mice were

immunized intraperitoneally with viable unmodified LNCaP cells at the specified intervals, followed by a final booster immunization with fresh prostate cells. Spleen cells from these mice were then fused with myeloma cells. Next, appropriate clones producing antibodies to the extracellular domain of PSMA were selected from those producing antibodies to other LNCaP-related antigens. This was accomplished by a screening process again using viable cells known to be PSMA-positive (i.e. LNCaP and prostate cells) or PSMA-negative (i.e. cultured normal kidney and colon cells). Those clones which were LNCaP+/cultured normal kidney -/ colon – and prostate + were further screened by the ability to immunoprecipitate and/or western blot a molecular species comparable to the 7E11 antibody as described in Examples 7 and 8. Reactivity with the external domain of PSMA was further confirmed as described in Example 9 (reactivity with viable LNCaP cells) and the results of the reactivity with normal and cancer tissues, is presented in Table 3.

In fact, Applicant used this same approach more than once to generate the four different antibodies described in the application. (**Bander Declaration #1, ¶7.**) Specifically, antibody E99 is derived from a completely different immunization/fusion experiment than the remaining antibodies denoted by the "J" prefix, i.e. J415, J533 and J591. (Id.) The fact that Applicant was able to use this procedure to obtain 4 different antibodies, with 2 completely separate immunization and fusion experiments, demonstrates that the disclosed procedure can be repeated and used to develop additional antibodies. Furthermore, procedures for sequencing the variable regions of J591 antibody, while not essential, are set forth in Example 12. Using these procedures, as well as the sequences themselves (which are suitable for hybridization assays), one of ordinary skill in the art would be fully able to identify additional binding portions. Accordingly, Applicant submits that the present application does provide enabling support to reproducibly obtain the antibodies of the claimed invention.

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B. The Antibodies of the Present Invention Bind to the Extracellular Domain of PSMA.

1. The Specification Provides Evidence that the Monoclonal Antibodies of the Present Invention Bind to the Same Antigen (although a different epitope) as the 7E11 Antibody (i.e. PSMA) and Bind to the Extracellular Domain of PSMA.

The Examiner's rejection on the grounds that the present application does not demonstrate that antibodies in accordance with the present invention bind to PSMA (the same antigen bound by 7E11) or an external region of PSMA is respectfully traversed. As noted at page 9, lines 3-9 of the specification, biochemical characterization and mapping have shown that the epitope or antigenic site to which the 7E11 antibody binds is present on the intracellular portion of the PSMA molecule. (**Exhibit 3:** Troyer et al., "Biochemical Characterization and Mapping of the 7E11-C5.3 Epitope of the Prostate-specific Membrane Antigen," Urol. Oncol., 1:29-37 (1995)) 7E11, as a result of its specificity for the intracellular domain, does not bind to viable LNCaP cells. (**Exhibit 4:** Troyer et al., "Location of prostate-specific membrane antigen in the LNCaP prostate carcinoma cell line," Prostate, 30:232-242. (1997)) Binding of the antibodies of the present invention to the same antigen as 7E11 and the external domain of PSMA is demonstrated by the examples of the present application. In Example 7, the immunoprecipitation and sequential immunoprecipitation studies confirm reactivity of E99, J415, J533 and J591 to PSMA. Specifically, the sequential immunoprecipitation study showed that these antibodies and 7E11 bind to the same molecule, i.e. PSMA. In addition, Example 8 of the present application shows that the 7E11 antibody and the antibodies of the present invention precipitate the same band in a Western Blot analysis. Since the 7E11 antibody is specific to PSMA, the claimed antibodies must also bind to PSMA. Moreover, the immunoflorescence against viable LNCaP cells described in Example 7 demonstrates that the antibodies bind to the external domain of PSMA. The results of Examples 9 and 11 as well as

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Figures 1-4 provide further support for binding of the antibodies of the present invention to the external domain of PSMA.

2. Further Evidence that the Monoclonal Antibodies of the Present Invention Bind PSMA, the Same Antigen Bound by 7E11.

The ability of the antibodies of the present invention bind to PSMA as described in the application is confirmed by the peer reviewed article Liu, et. al., "Monoclonal Antibodies to the Extracellular Domain of Prostate-specific Membrane Antigen also React with Tumor Vascular Endothelium," Cancer Res. 57:3629-34 (1997) ("Lui I") (attached hereto as **Exhibit 5**), which was senior authored by the inventor, Dr. Neil Bander. In Figure 1 on page 3630 of Liu I, a cross-immunoprecipitation experiment demonstrates that when one uses the 7E11 antibody to immunoprecipitate its antigen (i.e. PSMA) from LNCaP cells, and when this immunoprecipitated material is transferred to a Western Blot and probed with the antibodies of the present invention, those 4 antibodies react with the material immunoprecipitated by 7E11. The same is true in reverse – i.e., when a LNCaP lysate is immunoprecipitated individually by each of the 4 antibodies of the present invention, a Western Blot with 7E11 reveals that 7E11 is immuno-reactive with the immunoprecipitated species.

Figure 5 of Lui I provides further evidence of the ability of the antibodies of the present invention to bind the same PSMA antigen as 7E11. This figure demonstrates a competition binding assay comparing all 4 antibodies of the present invention to define which of these antibodies bind to the same or different epitopes of PSMA. Key to the issue here is that the technique, which is described on page 3630, first captures PSMA using the 7E11 antibody. The actual competition part of the assay is done after the capture step. This indicates that the 4 antibodies of the present

invention bind to PSMA captured by 7E11, again indicating all of these antibodies bind to the same molecule.

Moreover, it has been shown by investigators at Memorial Sloan-Kettering Cancer Center including Chang et al. that the PC3 cell line (which under normal circumstances is PSMA-negative) becomes immuno-reactive with 7E11 as well as the antibodies of the present invention after it has been transfected with the PSMA gene. (**Exhibit 6:** Chang et al., Cancer Res., 59:3192-3198 (1999)). And, Applicant performed a sequential immunoprecipitation analysis that confirms binding of PSMA by the antibodies of the present invention. ("Lui I" at page 3631.) This study demonstrates that a LNCaP lysate, when first "precleared" with any one of the antibodies of the present invention, is no longer immuno-reactive with 7E11. And again the converse is true.

3. Further Evidence that Monoclonal Antibodies of the Present Invention Bind to Extracellular Domain of PSMA

Lui I also confirms that the antibodies of the present invention bind to the external domain of PSMA. (See Lui I's immunoflourescence microscopy targeting of living prostate cancer cells (i.e. LNCaP cells) on pages 3632, left column, and Figure 3.) The ability of the antibodies of the present invention to bind to viable, non-permeabilized LNCaP cells (Figure 3, panels A,C,E and G) demonstrates that the binding occurs on the extracellular region of the PSMA molecule. On the other hand, the 7E11 antibody, which is known to bind to an intracellular epitope, does not bind to such viable cells (Figure 3, panel I). Only when LNCaP cells were permeabilized could the 7E11 antibodies (as well as the 4 antibodies of the present invention) react with these cells. Furthermore, as depicted in the immunoelectron micrograph of Figure 4 of Liu I, viable LNCaP cells were incubated with the J591 antibody of the present invention. As Figure 4 visually demonstrates, J591

localizes to the extracellular surface of the plasma membrane, (Panel A) whereas 7E11 demonstrates no binding (Panel B).

Further evidence that the antibodies of the present invention bind to the extracellular domain of PSMA is provided in Liu, et al., "Constitutive and Antibody-Induced Internalization of Prostate-Specific Membrane Antigen," Cancer Res. 58:4055-60 (1998) ("Lui II") (attached hereto as **Exhibit 7**) and also senior-authored by the inventor, Dr. Neil Bander. In particular, in Figure 1 of Lui II on page 4056, a time course study of the antibodies of the present invention show antibodies binding to viable LNCaP cells. In this experiment, the antibodies can first be seen binding to the plasma membrane and subsequently being internalized. Figure 3 of Lui II shows similar data at the level of the electron microscope.

Based upon all this evidence, it is clear that the antibodies of the present invention bind to an extracellular antigen of PSMA.

C. In vivo Data Demonstrates that the Antibodies of the Present Invention Successfully Target Tumor Tissue.

1. The Jain Factors are not Relevant

The Examiner questions the evidence presented that the antibodies of the present invention effectively target tumors *in vivo*. The Examiner cites Jain and three factors responsible for poor localization of macromolecules in tumors: (1) heterogeneous blood supply; (2) elevated interstitial pressure; and (3) large transport distances. Applicant respectfully submits that these three factors are not relevant to the clinical situation addressed by the present invention. First, prostate cancer predominantly involves the bone marrow and lymph nodes. These sites are, by definition, highly vascularized. (**Bander Declaration #2, ¶ 9.**) In addition, prostate cancer is unlike many other solid tumors in that its metastatic sites are small volume sites measure in microns or millimeters. (Id.)

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For these reasons, the tumor sites are very well supplied with antibody given the high levels of circulating antibody in the vascular compartment. Large transport distances, as a result, are not operative in this situation. (Id.) Similarly, interstitial pressure in these small volume sites within bone marrow and lymph nodes is also not operative. (Id.) Moreover, primary prostate cancers are also relatively small and multifocal and the factors recited by the Examiner are likewise not relevant. (Id.)

With respect to targeting the PSMA molecule on vascular endothelial cells, these three factors cited by the Examiner similarly are not operative. (**Bander Declaration #2, ¶10.**) Since blood supply is necessary to allow tumor nutrition and growth, and since the method of the invention directly targets blood vessels, the heterogeneity of the blood supply is not relevant. (Id.) Since in the method of the invention it is not required that the antibody extravasate into the tumor in the setting in which the vasculature itself is targeted, elevated interstitial pressure is not relevant. (Id.) And, of course, since again the method of the invention targets the blood vessels themselves and does not require antibody extravasation, large transport distances also is not relevant.(Id.)

2. Data

a. *In Vitro* Targeting Data

In addition to data presented in the application, further *in vitro* data demonstrating effective targeting (as well as ablation) of tumor cells using the antibodies of Applicant's invention is presented in Yang et al., AACR Abstract #2996 (1998) and a poster presentation presented by Ballangrud et al. **(Bander Declaration #2, ¶11).** These data are generated using LNCaP spheroids which are LNCaP cells that grow *in vitro* as tumor masses several hundreds of microns in diameter, rather than in a monolayer. These spheroid masses recapitulate an *in vivo* tumor mass to an extent and demonstrate the ability of the antibodies of Applicant's invention to penetrate into tumor masses.

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Indeed, these spheroids are substantially larger that the typical prostate cancer metastasis. (Id.) As shown in the data, the antibodies of Applicant's invention, conjugated with a florescein marker or isotope, are observed by confocal microscopy to penetrate into these tumor masses. (Id.) Beyond this penetration, use of the radioisotope antibody conjugate, shows that these relatively large tumor spheroids can be effectively destroyed or killed. (Id.) Also included in Exhibit B to the **Bander Declaration #2** is a series of graphs and photos which examine the volume of multiple spheroids over time, treated with and without dexamethasone and/or ^{90}Ytrium labeled specific (J591) or nonspecific (HuM195) antibody. (Id.)

b. *In Vivo* Targeting Data

In addition to the *in vitro* data mentioned above, *in vivo* animal data which demonstrates the ability of the antibodies of Applicant's invention to target tumor sites is appended in Exhibit C of the **Bander Declaration #2**. These 2 graphs examine Applicant's antibodies J591 and J415, as well as 7E11, conjugated to two different isotopes and demonstrate by quantitative analysis, that there is selective and specific uptake of radiolabeled antibody by PSMA–expressing tissues in an animal model (i.e. tumor xenografts). (**Bander Declaration #2, ¶12.**) More specifically, these graphs demonstrate that, over the 6-8 day period of observation, the relative amount of antibody in the tumor as compared to either blood or muscle continues to increase. (Id.) Not shown on these graphs is that an irrelevant antibody (B1) showed significantly lower tumor to non-tumor ratios than those found with these antibodies specific for PSMA. (Id.)

Clinical *in vivo* data demonstrating targeting of the antibodies of Applicant's invention to a non-prostate cancer in a human patient is appended as Exhibit D of the **Bander Declaration #2**. The subject patient had both hormone-refractory prostate cancer and biopsy-proven colon cancer that had spread (metastasized) to the liver. (**Bander Declaration #2, ¶13.**) The two photographs

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represent the patient's CAT scan and the patient's antibody scan. (Id.) The CAT scan shows sequential slices through the liver demonstrating the mass in the right lobe of the liver. (Id.) As shown in the antibody scan of this patient following administration of a radiolabeled antibody of Applicant's invention, there is intense uptake of radiolabel and therefore significant signal in the vasculature of the same liver metastasis, indicating antibody localization to this non-prostate cancer. (Id.)

c. The Antibodies of the Invention Effectively Ablate or Kill Tumor Tissue

As noted above, the antibodies of Applicant's invention have been shown to target tumor cells *in vivo* in both an animal model and the clinical setting. Moreover, as also discussed above, *in vitro* data using LNCaP spheroids demonstrates the ability of the antibodies of Applicant's invention to kill LNCaP cells growing in a tumor mass.

i. Further *In Vitro* Ablation/Killing Data

Data from an additional *in vitro* experiment appended in Exhibit E of **the Bander Declaration #2** demonstrate that the J591 antibody (both humanized and mouse) mediates antibody dependent cellular cytotoxicity (ADCC). (**Bander Declaration #2, ¶15.**) That is, human lymphocytes and anti-PSMA antibody will induce a lysis of human prostate cancer cells. (Id.) Controls in the studies consist of no antibody and no effector cells, and a humanized and mouse versions of an anti-leukemia ("irrelevant") antibody plus cells. (Id.) This is yet another mechanism by which the antibodies of the present invention demonstrate their cytotoxicity. (Id.) And as explained below, the antibodies of Applicant's invention are effective in killing tumor cells *in vivo* in both an animal model and the clinical setting.

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ii. *In Vivo* Ablation/Killing Data

The data appended in Exhibit F of the **Bander Declaration #2** shows that the use of a radiolabeled (^{213}Bismuth) antibody of Applicant's invention can delay and/or prevent tumor growth *in vivo* in an animal model. (**Bander Declaration #2, ¶16.**) In this animal study, the animals were inoculated with a human prostate xenograft of LNCaP cells and several days later were treated with ^{213}Bismuth conjugated J591 antibody. (Id.) Two control groups were studied. One group received no treatment whatsoever and the second group received ^{213}Bismuth conjugated to an irrelevant antibody which targets human leukemia cells but not prostate cancer cells. There were 6 animals per treatment group. (Id.) Figure 2 is the serum PSA (a prostate cancer antigen detectable in the serum of individuals with prostate cancer and an indicator of the presence/progression of prostate cancer) data of the mice shown in Figure 1. (Id.) In brief, the data shows that on day 51, the PSA levels in these mice is substantially higher in the two control groups than in the groups given anti-PSMA antibody conjugate. (Id.) This study demonstrates that there was a significant delay in the development of tumor growth in the anti-PSMA treated animals and half of those animals never developed detectable tumors. (Id.)

The first results of Applicant's recent experiments using antibody J591 conjugate in mice having large LNCaP xenograft tumors of approximately 1 cm in diameter, (this represents 5% of the animal's body weight) have shown similar xenograft killing effects. (**Bander Declaration #2, ¶17.**) Exhibit G of the **Bander Declaration #2** includes data from a number of different studies. Firstly, data labeled "G1", are results of a large series of animals treated with ^{131}Iodine-muJ591 (mouse J591) at different doses (100mCi or 300 mCi) and different routes of administration (intraperitoneal and intravenous). (Id.) The animals in these studies got a single treatment dose on day 0 approximately 10-14 days post tumor implantation, when tumors had reached approximately 1 cm in

diameter. Control animals received a single injection. Each line represents a growth curve for an individual tumor. (Id.) Exhibit "G2a" shows a point to point tracing of the average size of tumors in a group of animals (3-5 animals per group) treated with saline (PBS), J591 alone, J591 conjugated to a cytotoxic drug or animals treated with J591 conjugated to a different cytotoxic. (Id.) The same data appears in exhibit "Gb2" except that the curves are now "fitted" by a computer program. "Gb2" also indicates the number of animals in each group. (Id.) Exhibit "G2c" shows a plot of the weight of the animals in the different treatment groups showing that there was not significant adverse on the animals weight due to the treatment. However, the control (PBS) animals suffered the most with respect to weight as they became increasingly cachectic due to the increasing size of their tumors. (Id.) Exhibit "G3a" and "G3b" are graphs which show that the cytotoxic conjugates can effectively kill LNCaP cells *in vitro*. (Id.)

The data appended in Exhibit H of the **Bander Declaration #2** show that the use of radiolabeled antibodies and "naked" antibodies of Applicant's invention in a human patient are effective at both imaging/localization to non-prostate cancer and resulting in a measurable shrinkage of tumor. More specifically, this patient received humanized J591 antibody. The first 6 doses of antibody included a combination of "naked" antibody and antibody labeled with a trace amount of I^{131} which was for diagnostic purposes only and not intended as a therapeutic dose. Thereafter, this patient received 3 doses of purely "naked" antibody. The four photographs in Exhibit H are from the CAT scan and indicate nodal involvement in the neck, mediastinum, retroperitoneum and retrocrural area and pelvis. An additional photo is the patient's bone scan. Also included is a PET scan which demonstrates uptake of radiolabeled antibody in most of the areas shown on the CAT scan. The photograph labeled "H1" is the planar scan of the patient's antibody study which demonstrates uptake in the left neck node, mediastinum and retroperitoneum as well as pelvic nodes. This can

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also be seen on the SPECT study labeled "H2" where uptake in the neck node, mediastinum as well as the right shoulder (consistent with the increased uptake in the right shoulder on the bone scan). As of the date of the Bander Declaration #2, this patient had a 25% shrinkage of his measurable left neck mass and a 50% decline in his PSA.

III. The Section 102 Rejection

Claims 24-34 and 38-42 are rejected by the Examiner under 35 U.S.C. 102(e) as being unpatentable over Israeli et al., U.S. Patent 5,538,866. Applicant respectfully traverses this rejection. Claim 24 of the present application recites (as amended) a method of detecting normal, benign hyperplastic, and cancerous prostate epithelial cells using "an antibody or antigen binding portion thereof which binds to an extracellular domain of prostate specific membrane present as an integral membrane protein on a living cell." Israeli et al. does not teach such antibodies or binding portions thereof of the present invention and therefore does not constitute 102(e) art against the present application.

Israeli et al. purports to provide a "method to produce both polyclonal and monoclonal antibody using purified PSM antigens or polypeptides encoded by an isolated mammalian nucleic acid molecule encoding a mammalian PSM antigen." (column 6, lines 44-47) However, as argued successfully during prosecution of the parent application (Serial No. 08/838,682; issued as U.S. Patent No. 6,107,090) to the present application, in using synthetic amino acid sequences of PSMA as an immunogen to develop antibodies, one cannot be certain how well exposed such a peptide is nor how immunogenic it is. (**Bander Declaration #1,** paragraph 17 .) Furthermore, this does not take into account the 3 dimensional folding of the native molecule, nor its glycosylation or other post-translational modifications and other characteristics which are of significant importance in an antibody response. (**Id**.) An immunogenic peptide may also not be useful in the context of a heavily

glycosylated molecule such as PSMA. (**Id.**) Peptides or synthetic antigens encoded by an isolated mammalian nucleic acid molecule therefore, cannot effectively substitute for the natural tertiary and quarterary structure of a protein in a physiological situation. (**Id.**) Conversely, the method of the present invention, by using viable, unmodified prostate cancer cells, presents the native PSMA glycoprotein to the immunized animals immune system in a form whereby generated antibodies do recognize the native glycoprotein as it exits as an integral membrane protein of a living cell.

Moreover, there is no teaching in Israeli et al. of which part of the protein should be used to produce antibodies which will reliably detect the presence of the PSMA antigen as an integral membrane protein on the surface of a living cell. Such a determination would require undue experimentation. See In re Wands, 858 F.2d 731, 8 USPQ 1400 (Fed. Cir. 1988). This is particularly true with respect to antibodies to PSMA.

Horoszewicz, et al., "Monoclonal Antibodies to a New Antigenic Marker in Epithelial Cells and Serum of Prostatic Cancer Patients," Anticancer Res., 7:927-936 at 928 (1987) ("Horoszewicz") (attached hereto as **Exhibit 8**) noted that the indirect immunoperoxidase staining of formalin fixed (i.e., dead) LNCaP cells using 7Ell monoclonal antibody was positive. Staining of living LNCaP cells however, was negative. Moreover, the results in Wright et al., "Expression of Prostate Specific Membrane Antigen in Normal, Benign, and Malignant Prostate Tissue," Urol. Oncolog., 1:18-28 (1995) ("Wright') (attached hereto as **Exhibit 9**) taught that the PSMA epitope recognized by Mab 7Ell was localized to the cytoplasm. Wright states:

> Epitope-mapping experiments conducted in our laboratory have yet to demonstrate an epitope recognized by MAb 7E11-C5.3 in the extracellular domain of the PSMA glycoprotein (Troyer et al., unpublished results). As stated above, the only epitope recognized by MAb 7E11-C5.3 is located in the cytoplasmic domain. The sequence for this epitope is not found in the extracellular polypeptide region...

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Id. at 26. It was later concluded in Troyer I that the 7EII monoclonal antibody recognized an intracellular epitope. See also Liu as described above and Examples 7 and 9 of the present application. Moreover, despite the desire to develop an antibody to an extracellular domain for PSMA expressed in Troyer I, Applicant is not aware of any antibodies that have been made using a synthetic sequence of PSMA that will bind to viable prostate cancer cells. **Bander Declaration #1, ¶ 17.)** To the contrary, Applicant is aware of individuals who have tried but failed at such an approach. **(Id.)**

In particular, Troyer, et. al., "Location of Prostate-Specific Membrane Antigen in the LNCaP Prostate Carcinoma Cell Line," The Prostate 30:232-42 (1997) (attached hereto as **Exhibit 4**) states:

> It must be stated in any discussion of PSMA second-generation antibodies, however, that the mAb 7E11-C5.3 may be the most selective antibody for prostate tissue. It is highly likely that many of the mAbs generated against the extracellular domain of PSMA may be cross-reactive with other cellular antigens. Therefore, it may not be possible to greatly improve the sensitivity of PSMA imaging over that which is obtained using 7E11-C5.3.

Thus, the prior art's experience with antibodies to PSMA demonstrates that obtaining an antibody which binds to an external domain is hardly a matter of routine experimentation.

Moreover, the Examiner asserts that "although Israeli et al. is silent as to the internalization of bound antibody, the redistribution and internalization of surface antigens and antibodies bound to them is a well known art phenomenon" and cites Coleman et al., page 76, column 2, 3rd paragraph. Coleman however, is not applicable to the present invention. **(Bander Declaration #1, ¶16.)** Coleman describes the process of "capping" and "pinocytosis," and relates directly to cells of hematopoietic lineage, not of epithelial origin such as prostate cancer. (Id.). One cannot freely extrapolate observations on hematopoietic cells to epithelial cells. (Id.) Indeed, the specific mechanism of internalization described by Coleman is entirely different from the internalization

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mechanism which has been defined with respect to antibody binding to the extracellular domain of PSMA. (Id.) In particular, Coleman points out the requirement for bivalent or multivalent antibodies, indicating that monovalent antibodies are incapable of inducing internalization. By contrast, Applicant has found that even monovalent antibodies derived from those of the present invention are internalized. (Id.) Lastly, Coleman describes cells normally losing surface determinants in the internalization process with later re-expression of these determinants. In the present invention, loss of surface antigens has never been demonstrated, and indeed, regardless of the presence of absence of antibody, the extracellular domain of PSMA is continuously present. (Id.). Based on the foregoing, Applicant respectfully asserts that the Examiner's rejection based on Israeli et al., should be withdrawn.

IV. The Section 103 Rejection

Claims 24-34 and 37-42 are rejected by the Examiner under 35 U.S.C. 103(a) as being unpatentable over Israeli et al., U.S. Patent 5,538,866, in view of Schlom, Molecular Foundations of Oncology. Applicant respectfully traverses this rejection.

Schlom is cited by the Examiner for teaching "engineered antibody modifications and labeling techniques which are now standard in the art, and which are useful for purposes of increase effectiveness and decreased antigenicity." Schlom however, does not overcome the above-noted deficiencies of Israeli et al. and accordingly, Applicant respectfully asserts that the rejection based upon the combination of Israeli and Schlom should be withdrawn.

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In view of the foregoing, Applicant submits that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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Dated: March 12, 2001

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

For the paragraph beginning on page 12, line 29 (brackets indicate deletions and double dashes indicate insertions):

Figure 8 is a comparison of the heavy chain of monoclonal antibody J591 with the consensus sequence for Mouse Heavy Chains Subgroup IIA --(designated SEQ ID No. 20) --.

For the paragraph beginning on page 13, line 5:

Figure 11 is a comparison of the kappa light chain of monoclonal antibody J591 with the consensus sequence for Mouse Kappa Chains Subgroup V --(designated SEQ ID No. 21) --.

For the paragraph beginning on page 42, line 10:

The J591 VH is in Mouse Heavy Chains Subgroup IIA --(designated SEQ ID No. 20)-- (Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services (1991) ("Kabat"), which is hereby incorporated by reference). The sequence of J591 VH is compared to the consensus sequence for this subgroup in Figure 8.

For the paragraph beginning on page 45, line 4:

J591 VK is in the Mouse Kappa Chains Subgroup V --(designated SEQ ID No. 21)-- (Kabat, which is hereby incorporated by reference). The sequence of J591 VK corresponding to the ten identical clones is compared to the consensus sequence for the subgroup in Figure 11.

CLAIM SHEETS MARKED UP TO SHOW CHANGES

24. (Twice amended) A method of detecting normal, benign hyperplastic, and cancerous prostate epithelial cells or a port6ion thereof in a biological sample comprising:

providing an antibody or antigen binding portion thereof [a biological agent] which binds to an extracellular domain of prostate specific membrane antigen present as an integral membrane protein on a living cell, wherein the antibody or antigen binding portion thereof [biological agent] is bound to a label effective to permit detection of said cells or a portion thereof upon binding of the antibody or antigen binding portion thereof [biological agent] to said cells or a portion thereof;

contacting the biological sample with the antibody or antigen binding portion thereof [biological agent] having a label under conditions effective to permit binding of the antibody or antigen binding portion thereof [biological agent] to the extracellular domain of prostate specific membrane antigen of any of said cells or a portion thereof in the biological sample; and

detecting a presence of any of said cells or a portion thereof in the biological sample by detecting the label.

26. (Amended) A method according to claim 24, wherein the antibody or antigen binding portion thereof [biological agent] is internalized with the prostate specific membrane antigen.

27. (Amended) A method according to claim 24, wherein said contacting is carried out in a living mammal and comprises:

administering the antibody or antigen binding portion thereof [biological agent] to the mammal under conditions effective to permit binding of the antibody or antigen binding portion

thereof [biological agent] to the extracellular domain of the prostate specific membrane antigen of any of said cells or a portion thereof in the biological sample.

32. (Amended) A method according to claim 27, wherein the antibody or antigen binding portion thereof [biological agent] is internalized with the prostate specific membrane antigen.

34. (Amended) A method according to claim 24 [25], wherein [an antibody is used in carrying out said method,] said antibody is [being] selected from the group consisting of a monoclonal antibody and a polyclonal antibody.

37. (Amended) A method according to claim 24 [25], wherein an antigen binding portion of an antibody is used in carrying out said method, the binding portion being selected from the group consisting of an Fab fragment, an F(ab')2 fragment, and an Fv fragment.

40. (Amended) A method according to claim 24, wherein the antibody or antigen binding portion thereof [biological agent] is in a composition further comprising a physiologically acceptable carrier, excipient, or stabilizer.

41. (Amended) A method according to claim 24, wherein the antibody or antigen binding portion thereof [biological agent] is in a composition further comprising a pharmaceutically acceptable carrier, excipient, or stabilizer.